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Cytotoxic cutaneous adverse drug reactions during anti-PD-1 therapy

Goldinger, Simone M ; Stieger, Pascale ; Meier, Barbara ; Micaletto, Sara ; Contassot, Emmanuel ;
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Abstract: **PURPOSE:** Immunotherapy experienced impressive progresses in cancer treatment. Antibodies against PD-1 improved survival in different types of cancer including melanoma. They are generally well tolerated. However, skin toxicities including pruritus, rashes and vitiligo are reported. Although frequent, they have not been further characterized yet. In this analysis we aimed to systematically assess and characterize the adverse cutaneous reactions observed in melanoma patients treated with anti-PD-1 antibodies. **EXPERIMENTAL DESIGN:** Melanoma patients were treated with anti-PD-1 antibodies within clinical trials and early access program. Adverse cutaneous eruptions emerged in our melanoma patient cohort were systematically investigated and classified using histology and gene expression profiling in comparison to maculopapular drug rash, cutaneous graft versus host disease and the severe drug eruption toxic epidermal necrolysis. **RESULTS:** Between Feb 2013 and Sept 2015, 68 stage IV melanoma patients were treated at the University Hospital Zurich; 15 patients (22%) developed cutaneous reactions and 10 (15%) vitiligo. The cutaneous reactions ranged from small erythematous papules with mild pruritus to disseminated erythematous maculopapular rashes without signs of epidermal involvement to severe maculopapular rashes including epidermal detachment and mucosal involvement. Although skin involvement varied from mild rash to bullous drug eruptions, gene expression profiling pathogenically classified all investigated cases as toxic epidermal necrolysis-like reactions. **CONCLUSIONS:** As predicted by the PD-1 knock out mouse, anti-PD-1 antibodies frequently cause adverse cutaneous reactions. Gene expression profiling reminds in all cases to a toxic epidermal necrolysis-like pattern suggesting that PD-1/PD-L1 interaction is required to preserve epidermal integrity during inflammatory skin reactions.

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Cytotoxic cutaneous adverse drug reactions during anti-PD-1 therapy

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Statement of translational relevance

Antibodies against PD-1 have revolutionized cancer immunotherapy. This article describes relevant skin toxicities emerging upon treatment with antibodies against PD-1 such as nivolumab and pembrolizumab. Skin toxicities were classified using histology and gene expression profiling in comparison to mild maculopapular drug rashes and severe drug eruptions such as toxic epidermal necrolysis. Although the clinical picture was variable ranging from mild to severe gene expression profiling resembled in all cases to severe cytotoxic SJS/TEN-like patterns. This suggests that the PD-1/PD-L1 interaction is involved in the preservation of epidermal integrity during inflammatory skin reactions. We advocate a careful examination of the skin of patients treated with immunotherapy in the near future as these adverse cutaneous reactions can imply a loss of epidermal integrity.

Abstract

Purpose:

Immunotherapy experienced impressive progresses in cancer treatment. Antibodies against PD-1 improved survival in different types of cancer including melanoma. They are generally well tolerated. However, skin toxicities including pruritus, rashes and vitiligo are reported. Although frequent, they are have not been further characterized yet. In this analysis we aimed to systematically assess and characterize the adverse cutaneous reactions observed in melanoma patients treated with anti-PD-1 antibodies.

Experimental Design:

Melanoma patients were treated with anti-PD-1 antibodies within clinical trials and early access program. Adverse cutaneous eruptions emerged in our melanoma patient cohort were systematically investigated and classified using histology and gene expression profiling in comparison to maculopapular drug rash, cutaneous graft versus host disease and the severe drug eruption toxic epidermal necrolysis.

Results:

Between Feb 2013 and Sept 2015, 68 stage IV melanoma patients were treated at the University Hospital Zurich; 15 patients (22%) developed cutaneous reactions and 10 (15%) vitiligo. The cutaneous reactions ranged from small erythematous papules with mild pruritus to disseminated erythematous maculopapular rashes without signs of epidermal involvement to severe maculopapular rashes including epidermal detachment and mucosal involvement. Although skin involvement varied from mild rash to bullous drug eruptions, gene expression profiling pathogenically classified all investigated cases as toxic epidermal necrolysis-like reactions.

Conclusions:

As predicted by the PD-1 knock out mouse, anti-PD-1 antibodies frequently cause adverse cutaneous reactions. Gene expression profiling reminds in all cases to a toxic epidermal necrolysis-like pattern

suggesting that PD-1/PD-L1 interaction is required to preserve epidermal integrity during inflammatory skin reactions.

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Introduction

Antibodies against PD-1, a checkpoint in the effector phase of cytotoxic T cells, have been successfully used in cancer immunotherapy(1-3). Through the binding to PD-1, its ligands, namely PD-L1 and PD-L2, inhibit T cell-mediated immune responses. By preventing the binding of PD-L to PD-1, the FDA-approved antibodies pembrolizumab and nivolumab promote T cell-mediated cytotoxic responses which result in tumor regression in a variety of cancers(2). In several randomized pivotal studies pembrolizumab and nivolumab demonstrated improved overall response rates and progression-free survival compared to chemotherapy or the anti-CTLA-4 antibody ipilimumab(4-7).

Besides efficacy, the introduction of anti-PD-1 antibodies led to a new benchmark for treatment tolerability in cancer being a treatment with very few adverse events. Adverse cutaneous reactions have however been reported. These include skin exanthemas with a frequency of 10-25% and vitiligo in 9-11% of the treated patients(2, 4, 5). Robert et al. hypothesize that vitiligo is associated with a favorable outcome of anti-PD-1 therapy(8). Moreover, one case of bullous pemphigoid in association with pembrolizumab(9) and one case of psoriasiform exanthema under nivolumab(10) have been published. The adverse cutaneous reactions observed under the treatment with anti-PD-1 antibodies have not been characterized in detail so far.

Stevens-Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous drug eruptions characterized by widespread macules, papules and/or targetoid lesions with varying degrees of epidermal necrolysis clinically presenting as skin detachment and/or bullous skin lesions with mucosal erosions(11). Common drugs inducing SJS/TEN include allopurinol, trimethoprim-sulfamethoxazole, carbamazepin, lamotrigine, nevirapin, NSAIDs, phenobarbital and phenytoin(12). The molecular events that cause potentially fatal SJS and TEN are only partially understood, although "drug"-specific adaptive immune responses in the effector phase of TEN are well documented(13). In the skin, T cell-mediated immune responses occurring during SJS/TEN result in a massive keratinocyte apoptosis mediated by cytolytic molecules including FasL(14), perforin/granzyme B(15), annexin A1(16) and granulysin(17). Histologically SJS and TEN are characterized by full-thickness epidermal necrolysis due to extensive keratinocyte apoptosis associated with varying degrees of inflammation and epidermal infiltration by CD8+ lymphocytes.

In this study we aimed to systematically assess and characterize the adverse cutaneous reactions observed in advanced melanoma patients treated with anti-PD-1 antibodies using gene expression profiling and compared the data to other clinical types of drug eruptions.

Patients and Methods

Advanced melanoma patients were treated with either the anti-PD-1 antibody pembrolizumab (2mg/kg or 10mg/kg) or with nivolumab (3mg/kg) within clinical trials (clinicaltrials.gov NCT01704287, NCT01721746 and NCT02156804)(4, 7) and early access program. The first 68 melanoma patients treated with pembrolizumab or nivolumab at the University of Zurich were systematically investigated for adverse skin reactions by board certified dermatologists experienced in immunotherapy and familiar with associated adverse events (SMG and/or RD).

Complete and accurate skin examinations were performed before and during immunotherapy. Selected previously untreated skin eruptions of 8 patients emerging during therapy were biopsied and analyzed by histology. The decision to take a biopsy was based on whether the lesion was new (i.e. not apparent prior treatment start), on the severity (grade 2 or greater), on the localization and distribution (favoring multiple and disseminated lesions) and on the clinical characteristics (palpable or scaling presentation). In general, the most infiltrated lesion was chosen and biopsied provided patient's consent and that the lesion was in a region of the body that allowed proper wound healing. Expression of PD-1 on skin infiltrating T-cells (eBioscience, San Diego, CA) and PD-L1 (Immunobiology Yale, New Haven, CT) on keratinocytes at the foci of lymphocytic epidermal infiltration was assessed by immunohistochemistry.

Gene expression analysis of 5 out of these 8 skin biopsies during anti-PD-1 therapy with pembrolizumab were in addition analyzed and compared to expression profiles of skin biopsies from patients with maculopapular rashes (MPR) (n=8), SJS/TEN (n=5), and cutaneous graft-versus-host disease (GVHD) (n=9). RNA was isolated from patients skin (n=5) and from healthy control skin (n=4) using a Qiagen RNeasy kit (Qiagen) following manufacturer's instructions, and total RNA was converted into cDNA by standard reverse transcription using a RevertAid RT Reverse Transcription Kit (Thermo Scientific). Quantitative PCR was performed using Power SYBR Green PCR Master Mix

(Applied Biosystems). Primer sequences were obtained from <http://pga.mgh.harvard.edu/primerbank/>. The real-time PCR included an initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 56°C for 1 min, 72°C for 1 min, and one cycle of 95°C for 1 min, 56°C for 30 s, 95°C for 30 s. Moreover, we performed gene expression arrays (Affymetrix) on skin of MPR patients (n=8), SJS/TEN patients (n=5), GVHD patients (n=9) and healthy donors (n=8) as controls. Healthy skin was obtained from healthy individuals undergoing plastic surgery. All human skin biopsies were collected with informed written consent upon approval of local ethical committees and were conducted according to the Declaration of Helsinki Principles (KEK Nr. 647). Based on the results of the gene expression arrays, we selected a set of genes that could be used as specific signature for TEN as opposed to MPR and GVHD (Figure 2A). Using quantitative RT-PCR we determined the expression levels of the gene set specific for SJS/TEN in the skin of patients with an adverse cutaneous reaction occurring during anti-PD-1 therapy.

Results

Between Feb 2013 and Sept 2015, 68 patients were treated with either pembrolizumab 2mg/kg, 10mg/kg (every three weeks), or nivolumab 3mg/kg (every two weeks) in different clinical trials (clinicaltrials.gov NCT01704287, NCT01721746 and NCT02156804)(4, 7) and early access program. Out of the first 68 stage IV melanoma patients treated with pembrolizumab (n=54) or nivolumab (n=14) at the University Hospital of Zurich 15 patients (22%) developed any cutaneous inflammatory reaction and 10 (15%) a vitiligo. Clinically, the adverse cutaneous reactions ranged from small erythematous papules with mild pruritus to disseminated erythematous maculopapular rashes without major clinical signs of epidermal involvement to severe maculopapular rashes including epidermal detachment and mucosal involvement (Table 1). Histology performed on selected lesional skin biopsies of 8 patients at onset of the adverse cutaneous reaction revealed different manifestation grades of apoptotic keratinocytes and even focal full thickness necrosis of the epidermis in two cases in the histology (Table 1). In particular, a 77 year-old patient presented with a generalized unspecific maculopapular rash predominantly affecting the trunk with focal areas of epidermal detachment a few days after the first pembrolizumab infusion (Figure 1A, <10% of the body surface area). Histology showed epidermal damage with apoptotic keratinocytes, subepidermal lymphocytic infiltrates and dermo-epidermal

cleavage (Figure 1B). One week later, mucosal involvement and genital ulcerations developed. Taken together these features led to a diagnosis of SJS, and pembrolizumab therapy was discontinued.

Skin biopsies from six patients were also analyzed by immunohistochemistry and revealed in all cases an accumulation of CD8+ cells at the dermo-epidermal junction and some CD8+ exocytosis within the epidermis, as well as keratinocyte apoptosis suggestive of a cytotoxic etiology. Expression of PD-1 on skin infiltrating T-cells and keratinocytes at the foci of lymphocytic epidermal infiltration was also assessed by immunohistochemistry (Figure 1C/1D). Despite topical steroids, the lesions of this patient subsequently evolved into persistent polygonal flat erythematous papules, clinically suggestive of lichen planus (Figure 1E). Accumulation of cytotoxic CD8+ lymphocytes in the junction zone and in the epidermis causing apoptotic cell death of keratinocytes, classical for lichen planus, was confirmed by histology (Figure 1F). PD-L1 expression on keratinocytes was clearly detectable by immunohistochemistry in the proximity of T-cells (Figure 1G/1H).

In all cases, none of the concomitant medications taken by patients had been recently started or were known to cause SJS/TEN (Table 1). Furthermore, lymphocyte transformation tests to the concomitant medication taken by the patient 1 did not provide evidence for a "drug"-specific immune response (data not shown) (Table 1).

Treatment included systemic steroids (prednisone 1mg/kg, tapered for 4 weeks), systemic steroids (over a course of 4 weeks), disinfectant agents when necessary, and re-hydration of the skin. Treatment with pembrolizumab could be continued in 4/6 cases and did not further affect the skin.

Gene expression profiling of RNA extracted from lesional skin of validated cases of MPR (8 cases), SJS/TEN (5 cases) and cutaneous GVHD (9 cases) enabled us to identify a set of 18 genes for which the expression levels enable differentiation between the three diagnostic categories (Figure 2A). Analysis of the level of expression of these 18 genes was performed in the lesional skin biopsies of 5 patients, and revealed a gene expression profile reminiscent of SJS/TEN (Fig 2B). Both SJS/TEN skin (n=5) and skin of 5 patients presenting an adverse cutaneous reaction upon anti-PD-1 therapy had significant upregulation of *Elafin*, *SPRR2B*, *Granzyme B*, *CXCL9*, *CXCL10* and *CXCL11* whereas expression of *Desmocollin 3*, *Loricrin*, *Filaggrin* and *Keratin 1* were similar to those in healthy skin.

Differences were seen in the expression levels of *CCL27*, *NURR1*, *GNLY*, *FasL* and *Perforin* which were all upregulated in the skin of the 5 anti-PD-1 patients but not in SJS/TEN skin (Figure 2B).

Taken together our clinical, histological, immunohistological and gene expression analyses provide evidence that the adverse cutaneous reactions observed in patients treated with anti-PD-1 antibodies is reminiscent of SJS/TEN, and points to a role for PD-1 in regulating cytotoxic T-cell responses in the skin.

Discussion

The crosstalk between cancer cells and immune cells of the tumor microenvironment is crucial for the outcome of anti-tumor immune responses and immunotherapy. In various cancers, these interactions often result in a local immunosuppression resulting in the escape of tumor cells from immune-surveillance. The use of checkpoint inhibitors such as antibodies to PD-1 leads to significant clinical benefits by inducing advanced and metastatic tumor regression. Although anti-PD-1 antibody therapy is safe and well tolerated in melanoma patients(2, 18-20), adverse cutaneous reactions have been reported(2, 4, 9, 10, 21). Here, we report and describe adverse cutaneous reactions during anti-PD-1 immunotherapy with pembrolizumab. In 22% of the patients, which is more than reported in clinical trials(4, 7, 21), we observed inflammatory skin lesions ranging from mild maculopapular rashes, typically associated with scaling and/or lichenoid lesions to very severe SJS-like skin lesions that did slowly improve and resulted in a chronic lichen planus.

Clinical and histological features of the lesions strikingly resemble the findings reported in mice with a disrupted PD-1 gene(22). These mice develop a lupus-like inflammatory syndrome with proliferative glomerulonephritis, arthritis with sometimes granulomatous inflammation and skin lesions- reported as “dermatitis-like lesions, necrotic lesions and erythema”(22). The histologies of the skin of these mice present features compatible with lichen planus including acanthosis, hypergranulosis and apoptotic keratinocytes together with a lymphocytic infiltrate of the grenz zone of the basal membrane resulting in a vacuolization and sometimes split formation(22).

The histological analysis of these human adverse cutaneous reaction cases demonstrated signs of a cytotoxic skin eruption characterized by an accumulation of CD8+ T-cells at the dermo-epidermal

junction and CD8+ T-cell exocytosis into the epidermis with apoptotic keratinocytes. These features can also be observed in severe immune-mediated skin diseases such as acute GVHD and SJS/TEN. Gene expression analysis of lesional skin from anti-PD-1-treated patients revealed a gene expression profile resembling SJS/TEN with an upregulation of major inflammatory chemokines such as CXCL9, CXCL10 and CXCL11, of cytotoxic mediators such as Perforin and Granzyme B and the pro-apoptotic molecule FasL, as well as an upregulation of PD-L1 which was confirmed by immunohistochemistry in 3 cases. In contrast, the expression pattern of selected genes in the skin lesions of anti-PD-1-treated patients was different from that seen in acute GVHD and MPR. Therefore, clinical, histological, immunohistological, and gene expression analyses strongly suggest that, at least in some patients, anti-PD-1 antibody can induce SJS/TEN-like adverse cutaneous reactions.

The intensity of the immune mediated tissue damage varies and is interindividual - a possible explanation could be the genetic predisposition and variation based on single nucleotide polymorphisms in genes related to immune functions. These genetic background alterations can cause differences in the susceptibility to develop cutaneous drug reactions.

The exact pathomechanism of the adverse cutaneous reactions occurring upon pembrolizumab and nivolumab therapy remains to be elucidated. While vitiligo can be considered as a successful (re)-activation of T cells with a repertoire specific to melanocyte antigens, the induction of a cytotoxic response to keratinocytes was not expected, and is indicative of the activation of T cells with non-melanoma-derived self-antigen specificity(ies). Interestingly, both vitiligo and/or cutaneous reactions emerging during nivolumab treatment in melanoma patients have recently been reported to be associated with overall survival(23). As a consequence, cutaneous reactions during anti-PD-1 treatment could potentially be used as biomarkers in the therapy. Although larger prospective analyses are still needed to validate this association, detection and diagnosis of cutaneous reactions during anti-PD-1 therapy gain further importance in this context. By inhibiting T cell activation and sustaining Tregs(24), the PD-1/PD-L pathway plays a major role in peripheral tolerance including transplant(25) and feto-maternal tolerance(26). The concept of a tolerogenic role for PD-1/PD-1L has emerged from observations that PD-1-deficient mice develop autoimmune pathologies(27) including lichenoid reactions(22). One could hypothesize that, at the steady state, PD-1/PD-L interactions are crucial for the homeostasis of T cells in the skin and for preventing severe skin-directed inflammatory reactions

from occurring. In line with this, it has been recently reported that, in a mouse model that PD-L1 expressed on keratinocytes presenting self-antigens, regulates autoreactive CD8⁺ T cell activity, and prevents the development of cutaneous autoimmune disease(28). The reason for which cutaneous adverse events in anti-PD-1-treated patients can vary from vitiligo to SJS-like reactions remains unknown and larger series of subjects would be required to assess this. A detailed characterization of the T cells causing damage to healthy tissues in patients treated with anti-PD-1 antibodies as well as complementary skin investigations in patients without adverse skin reactions would be of interest for a better understanding and, ultimately, the prevention of such severe forms of adverse cutaneous reactions.

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Figure and Table legends:

Table 1. Patients' characteristics including clinical and histological presentation.

Figure 1. Clinical and histological presentation of a metastatic melanoma patient developing a severe adverse cutaneous reaction (ACR) upon treatment with pembrolizumab. a-d. Stevens-Johnson Syndrome (SJS), e-h. Lichen planus.

a. Clinical features of SJS; **b.** Histology of SJS (hematoxylin-eosin stain); **c.** PD-1 stain of SJS; **d.** PD-L1 stain of SJS. **e.** Clinical features of lichen planus; **f.** Histology of lichen planus; **g.** PD-1 stain of lichen planus; **h.** PD-L1 stain of lichen planus.

Figure 2. Gene expression profiling. a The mRNA expression levels of 6564 different genes were measured in lesional skin of MPR (n=8), Lyell (n=5) and GVHD (n=9) patients and skin of healthy donors (n=8) using the Affymetrix Human Transcriptome Array 2.0. The relative expression levels were normalized to healthy skin and indicated as fold change ($2^{-\Delta\Delta C_t}$). **b.** The mRNA expression levels of selected genes were measured in lesional skin of patients developing a skin drug eruption under anti-PD-1 therapy (n=5). The relative expression levels were normalized to healthy skin (n=4) and indicated as fold change ($2^{-\Delta\Delta C_t}$). Statistical analyses were performed using the student's t-test. * indicates a p-value ≤ 0.05 , ** indicates a p-value ≤ 0.01 , *** indicates a p-value ≤ 0.001 , **** indicates a p-value ≤ 0.0001 .

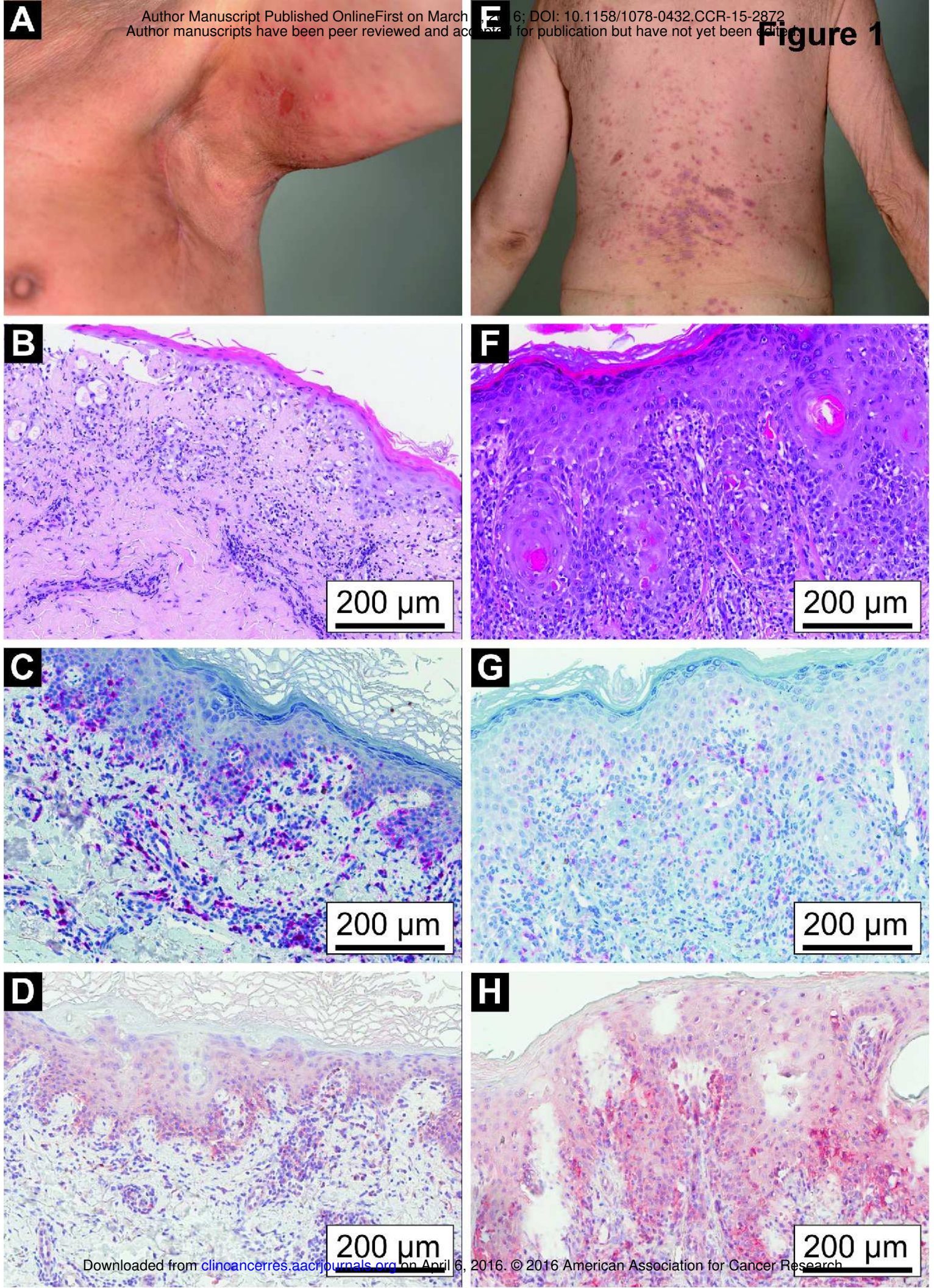


Figure 2

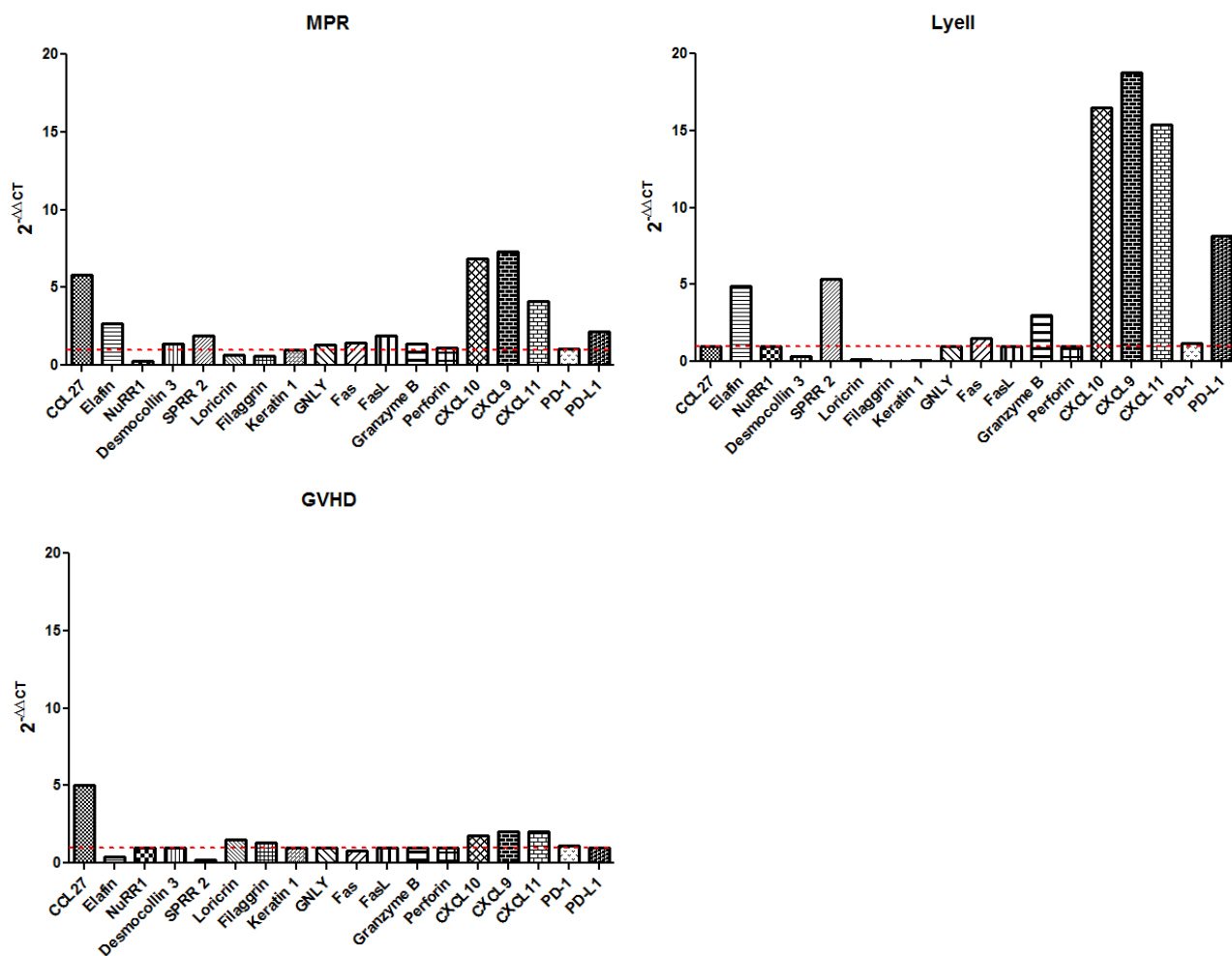
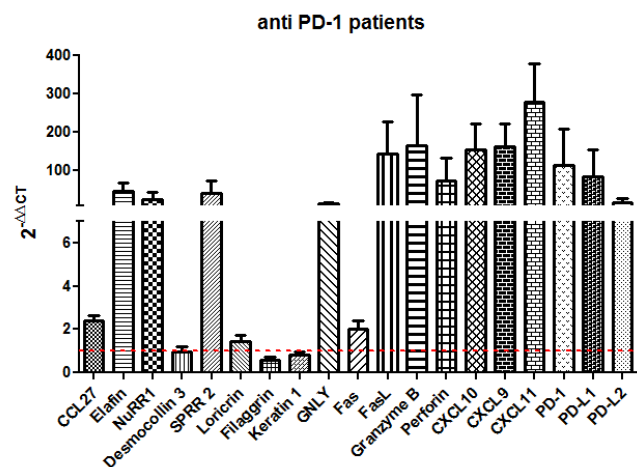
a**b**

Table 1. Patients' characteristics, Clinical Presentation and Histology of the adverse cutaneous reactions

	Characteristics (m/f; age (y))	Onset (weeks after starting anti-PD-1 abs)	Clinical Presentation	Histology	Concomittant medication	Other possibly treatment related adverse reactions	Discontinuation of pembrolizumab (yes/no)	Treatment of the adverse cutaneous drug reaction
Patient 1	m; 76	1 (after 1 infusion)	Maculopapular rash evolving into subepidermal blister formation including mucosal involvement with pain and pruritus, evolving into lichen planus	4 biopsies over time: 1. Lichenoid dermatitis with accumulation of CD8+ cells at the dermo-epidermal junction and CD8+ exocytosis and focal keratinocytic apoptosis 2. Lichenoid inflammation with vacuolization of junction zone and keratinocytic apoptosis 3. Lichenoid accumulation of cytotoxic CD8+ lymphocytes in the junction zone and in the epidermis with apoptotic keratinocytes 4. Lichenoid inflammation with hyperkeratosis resembling lichen verrucosus	Phenprocoumon; Spironolactone; Acetylsalicylic acid; Bisoprolol; Metamizole*; Rabeprazole; Mitrazapine; Lorazepam; Torasemide, Ramipril; Oxycodone*; Dalteparin**	none	yes	Systemic Prednisone 1mg/kg (tapering for 4 weeks); Topical Steroids, including oral application for 4 weeks; Topical disinfectants (Panthenol, Hyaluronate Sodium with Silver Sulfadiazine; Chlorhexidine) Skin hydration
Patient 2	m; 66	6 (after 3 infusions)	Disseminated maculopapular rash with moderate pruritus	Lichenoid drug reaction with follicular accentuation focal spongiosis and CD8+ exocytosis	Fluticasone/Salmeterol; Salbutamol	Vitiligo	no	Topical steroids for 4 weeks; Skin hydration
Patient 3	m; 50	5 (after 3 infusions)	Disseminated maculopapular rash with main focus on the trunc and neck. No pruritus.	Lichenoid dermatitis with vacuolization of junction zone and focal keratinocyte apoptosis	Pantoprazole***; Bisoprolol***	Anaemia (haemolysis)	yes (due to anaemia)	Topical steroids for 4 weeks; Skin hydration
Patient 4	m; 82	1 (after 1 infusion)	Small erythematous papules with moderate pruritus	Lichenoid dermatitis with CD8+ cells in the dermo-epidermal junction and focal full thickness necrosis	Phenprocoumon	none	no	Skin hydration
Patient 5	m; 72	51 (after 17 infusions)	Psoriasiform disseminated skin lesions predominantly on the lower extremities both sides. Mild pruritus	2 biopsies over time: 1. Focal acanthosis and spongiosis and some apoptotic keratinocytes with lichenoid aspect 2. Lymphocytic infiltration of the adnexa	Simvastatin; Losartan; Chondroitin sulfate	Vitiligo	no	Topical steroids for 4 weeks; Skin hydration
Patient 6	m; 78	21 (after 7 infusions)	Focal erythematous plaques on the trunk and on the neck with mild pruritus	Lichenoid drug reaction with lymphocytic infiltrate and few eosinophils	Candesartan; Acetylsalicylic acid; Atorvastatin	Vitiligo	no	Systemic Prednisone 1mg/kg (tapering over 2 weeks); Topical steroids for 4 weeks; Skin hydration
Patient 7	m; 58	9 (after 3 infusions)	Focal erythematous plaques on the lower leg with mild pruritus	Lymphocytic perivascular sleeve-like infiltrates and lichenoid inflammation with spongiosis	Lisinopril; Ibuprofen; Xylometazoline	Vitiligo	no	Topical steroids for 2 weeks; Skin hydration
Patient 8	f; 66	60 (after 21 infusions)	Focal erythematous papule with moderate pruritus on the back	Acanthosis, papillomatosis and lichenoid inflammation	Losartan	none	no	Skin hydration

* Start 8 weeks before onset
 ** Start 1 week before onset
 *** Start 4 weeks before onset

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